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Geographical susceptibility of Louisiana and Texas populations of the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) to *Bacillus thuringiensis* Cry1Ab protein

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Abstract

The susceptibilities of 18 field populations of the sugarcane borer, *Diatraea saccharalis* (F.), to two sources of *Bacillus thuringiensis* (Bt) Cry1Ab protein were determined using laboratory bioassays. Fifteen of the 18 field populations were collected from seven locations across Louisiana and the other three populations were sampled from the Gulf Coast area of Texas during 2004–2006. Neonates of *D. saccharalis* were exposed to a meridic diet treated with selected concentrations of Cry1Ab protein. Larval mortality was measured at 7 days after inoculation. Statistically significant differences in median lethal concentrations (LC_{50}s) were detected among insect populations from different geographical locations, but the field populations remained as susceptible as a laboratory strain of *D. saccharalis* that had been maintained in the laboratory for >20 years without exposure to any chemical insecticides or Bt toxins. The LC_{50}s of Cry1Ab protein, which was extracted from DKC69-70 Bt corn hybrid, ranged from 0.03 to 0.32 μg/g for the seven field populations collected during 2004. The LC_{50} values based on bioassays with purified, trypsin-activated Cry1Ab protein from a recombinant *Escherichia coli* culture were 0.03–0.17 μg/g for the 11 field populations collected during 2005–2006. Small changes in Cry1Ab susceptibility were detected among crops, years of sampling, or locations. All field-collected insect populations, except one, exhibited lower LC_{50} values than the laboratory strain. The results of this study suggest that field populations of *D. saccharalis* remain generally susceptible to the Cry1Ab protein after 8 years use of transgenic Bt corn in Louisiana and the Gulf Coast area of Texas.

Keywords: *Diatraea saccharalis*; Cry1Ab; *Bacillus thuringiensis*; Susceptibility; Field corn

1. Introduction

Evaluating the susceptibility of an insect pest species to insecticides across geographic areas is useful in assessing the potential risk of resistance development, understanding if variation in susceptibility among different populations is associated with the historical use of insecticides, and measuring the success of resistance management programs (United States Environmental Protection Agency, 2001). Significant variation in susceptibility to an insecticide among geographic populations of an insect species often

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indicates a high risk of resistance development (Kinsinger and McGaughey, 1979).

The sugarcane borer, *Diatraea saccharalis* (F.), is a key pest of sugarcane in the Americas (Long and Hensley, 1972; Reagan, 2001; Posey et al., 2006). Occasionally, this insect also causes economic losses in rice and grain sorghum (Castro et al., 2004a). In recent years, *D. saccharalis* has become an important corn stalk boring species in some areas in the mid-southern region of the United States, especially in Louisiana (Castro et al., 2004a; Huang et al., 2006a) and Texas (Porter et al., 2005). A 3-year survey (2004–2006) in Louisiana showed that *D. saccharalis* accounted for 73% of the total corn borer populations across the major corn areas of the state (Huang et al., 2006a). Large sugarcane borer infestations on field corn were also reported in south and central Texas during 2005 (Porter et al., 2005). This insect species was recently listed as a target pest of transgenic *Bacillus thuringiensis* (Bt) corn in the United States (United States Environmental Protection Agency, 2005a, b).

Although studies have shown that corn plants expressing Cry1Ab protein (e.g. YieldGard® corn) are less potent against *D. saccharalis* than against other major corn borer species such as European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* Dyar (Castro et al., 2004b; McAllister et al., 2004; Huang et al., 2006b), Bt corn has been successfully used since 1999 to control a corn borer complex of *D. grandiosella* and *D. saccharalis* in the mid-southern region of the United States (Castro et al., 2004a; Sankula and Blumenthal, 2004). Transgenic Bt corn is now the most important tool for management of corn borer pests in the region, accounting for >40% of the total corn acreage (National Agricultural Statistics Service, 2006; Huang et al., 2006a). The high adoption of Bt crops will place strong selection pressure on target pest populations that could eventually lead to resistance in the field. Management of *D. saccharalis* resistance, therefore, is important in order to ensure the long-term success of transgenic Bt corn technology for the region.

Although resistance to Bt in field populations has not resulted in field control failures in target insect species after 11 seasons of commercial use of Bt corn and Bt cotton, major resistance genes that could allow insects to complete development on Bt crops have been detected in three species targeted by Bt cotton (Gould et al., 1997; Tabashnik et al., 2000; Akhurst et al., 2003; Gunning et al., 2005; Xu et al., 2005) and one species, *D. saccharalis*, targeted by Bt corn (Huang et al., 2007a; Wu et al., 2007). Bt-resistant *D. saccharalis* demonstrated resistance to all seven commercial corn hybrids expressing the Cry1Ab protein evaluated by Wu et al. (2007).

Information regarding regional differences in susceptibility to Bt toxins in populations of *D. saccharalis* is unavailable. In this study, the susceptibilities of a laboratory strain and 18 field populations of *D. saccharalis* to two sources of Cry1Ab protein were determined using laboratory bioassays. These field populations were collected from corn, sugarcane, rice, and grain sorghum from seven locations in Louisiana and three locations of the Gulf Coast area of Texas. The objectives of this study were to estimate geographical variation in susceptibility of *D. saccharalis* to Bt proteins, and to provide baseline information to measure any notable changes in Bt susceptibility that could occur in field populations of this species following several years of commercial use of transgenic Bt corn.

2. Materials and methods

2.1. Insect collection

During 2004, seven field populations of *D. saccharalis* were established from pupae or late-stage larvae collected from four crops in five parishes in Northeast and Central Louisiana (Fig. 1 and Table 1). Three populations were

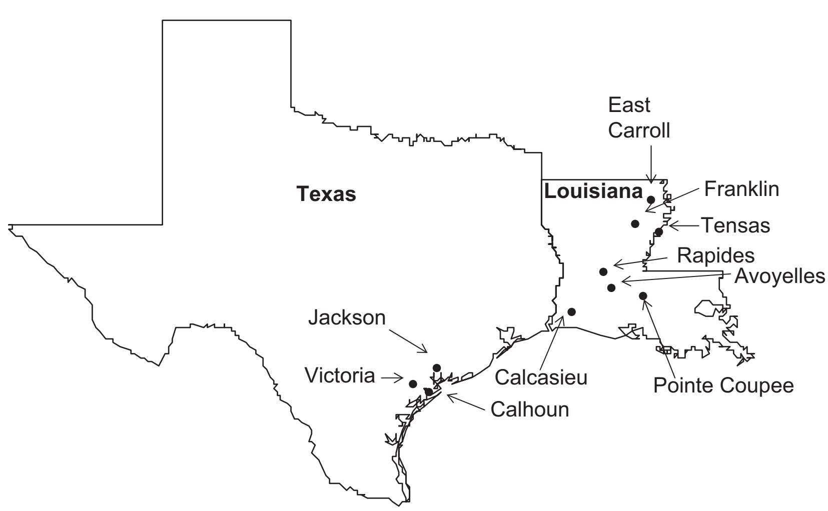

Fig. 1. Sampling locations (parish/county) where *Diatraea saccharalis* were collected in Louisiana and Texas, USA.

Table 1
Susceptibility of Louisiana populations of *Diatraea saccharalis* collected during 2004 to *Bacillus thuringiensis* Cry1Ab protein extracted from Bt corn leaf tissues

Location/ population (parish)	Host	Generation	No. of insects collected	N[a]	χ^2	p	Slope ± SE	LC_{95}(95%CI) (μg/g)[b,c]	LC_{50}(95%CI) (μg/g)[c,d]
Lab[e]	Sugarcane	>20	–	259	30.12	0.0255	1.94 ± 0.29	0.38(0.28–0.54) d	2.67(1.48–7.92) c
Franklin[e]	Corn	2	150	236	24.34	0.0417	2.99 ± 0.43	0.27(0.21–0.34) d	0.97(0.70–1.70) c
Rapides	Corn	1	61	336	37.63	0.0098	1.84 ± 0.25	0.15(0.11–0.20) b	1.18(0.73–2.63) c
Pointe Coupee	Corn	1	41	261	21.46	0.2567	3.57 ± 0.34	0.19 (0.16–0.21) c	0.54(0.43–0.74) b
Franklin	Sorghum	1	150	214	44.80	0.0001	3.20 ± 0.70	0.32(0.22–0.44) d	1.06(0.69–3.04) c
Franklin	Rice	2	150	267	12.58	0.8162	3.90 ± 0.40	0.23(0.20–0.26) cd	0.61(0.50–0.81) b
Avoyelles	Sugarcane	3	65	318	28.49	0.0550	3.22 ± 0.53	0.04(0.03–0.05) a	0.12(0.09–0.20) a
Calcasieu	Sugarcane	3	57	317	14.28	0.7109	2.44 ± 0.35	0.03(0.02–0.04) a	0.15(0.11–0.22) a

[a]Total number of neonates assayed.
[b]LC_{50} = 50% lethal concentration and 95% CI = 95% confidence intervals.
[c]LC values within a vertical column followed by different letters are significantly different, lethal dose ratio tests (Robertson and Preisler, 1992).
[d]LC_{95} = 95% lethal concentration and 95% CI = 95% confidence intervals.
[e]LC_{50} values of the two populations were reported in Huang et al. (2006b).

Table 2
Susceptibility of Louisiana (LA) and Texas (TX) populations of *Diatraea saccharalis* collected from field corn during 2005–2006 to trypsin-activated purified *Bacillus thuringiensis* Cry1Ab protein

Year	Location/ population (parish/county)	Generation	No. of insects collected	N[a]	χ^2	p	Slope ± SE	LC_{50} (95% CI) (μg/g)[b,c]	LC_{95} (95% CI) (μg/g)[c,d]
–	Laboratory	>20	–	577	11.37	0.8779	2.16 ± 0.19	0.12(0.10–0.14) f	0.70(0.55–0.98) d
2005	Franklin, LA	3	120	450	5.20	0.9985	2.77 ± 0.29	0.10(0.09–0.12) def	0.40(0.32–0.54) cd
	Rapides, LA	3	120	495	13.38	0.4970	2.02 ± 0.36	0.06(0.03–0.08) bc	0.39(0.30–0.60) cd
	Tensas, LA	3	85	512	8.13	0.8355	5.47 ± 0.48	0.09(0.08–0.10) cd	0.18(0.16–0.21) a
	East Carroll, LA	3	65	616	17.76	0.4716	3.91 ± 0.30	0.11(0.10–0.12) ef	0.30(0.26–0.36) bc
2006	Franklin, LA	1	150	462	25.38	0.1148	1.85 ± 0.19	0.09(0.08–0.11) cde	0.72(0.52–1.16) d
	Rapides, LA	2	150	445	10.46	0.6556	2.10 ± 0.39	0.03(0.01–0.04) a	0.16(0.12–0.23) a
	Tensas, LA	1	78	608	21.99	0.4602	1.93 ± 0.17	0.17(0.15–0.20) g	1.09(0.82–1.60) e
	East Carroll, LA	2	58	499	13.21	0.5099	3.40 ± 0.36	0.07(0.06–0.08) bc	0.21(0.18–0.26) ab
	Victoria, TX	1	30	768	17.80	0.7182	1.86 ± 0.19	0.08(0.06–0.09) bc	0.95(0.82–1.60) d
	Jackson, TX	1	25	619	41.20	0.0014	1.39 ± 0.27	0.04(0.01–0.06) ab	0.58(0.36–1.61) d
	Calhoun, TX	3	20	632	44.03	0.0006	2.00 ± 0.48	0.03(0.01–0.05) a	0.19(−.14–0.40) ab

[a]Total number of neonates assayed.
[b]LC_{50} = 50% lethal concentration and 95% CI = 95% confidence intervals.
[c]LC values within a vertical column followed by different letters are significantly different, lethal dose ratio tests (Robertson and Preisler, 1992).
[d]LC_{95} = 95% lethal concentration and 95% CI = 95% confidence intervals.

collected from corn, two from sugarcane, one from grain sorghum, and one from rice. The parental collections used to establish each population ranged from 41 to 150 individuals. During 2005–2006, 11 populations were established from pupae and larvae collected from cornfields in four parishes of Louisiana and three locations in Texas (Fig. 1 and Table 2). Each insect population established during 2005–2006 was initiated from 58 to 150 field-collected individuals for each of the eight Louisiana populations and from 20 to 30 individuals for each of the three Texas populations. Larval and pupal mortality could occur during insect rearing in the laboratory. The actual initial individuals that made contributions to each population could be less than the number of insects collected from the field. Based on independent data from rearing *D. saccharalis*, mortality of field-collected larvae reared on meridic diet was usually low (e.g. <9%) with a high pupal emergence rate (e.g. >95%) (Huang et al., 2007a).

Insects were collected during the first field generation (for the 2004 Franklin population from corn plants) or the second field generation (for all other insect populations).

In the sampling locations in Northeast Louisiana, corn is the predominant host of *D. saccharalis*, accounting for ≈82% of total host crop acreages. Bt corn has been planted on the maximum allowable acreage (i.e. 50%) in this area. Two other minor host crops are rice and grain sorghum, representing 15% and 3% of the total host crop acreages, respectively. Sugarcane is not a farm crop in this area (Louisiana AgCenter, 2005). In the sampling areas located in Central Louisiana, a mixture of sugarcane, corn, rice, and sorghum are planted, each representing approximately 36%, 17%, 29%, and 18% acreages of the host crops. The corn produced in the seven parishes sampled in Louisiana accounted for approximately 40% of the total corn production of the state (Louisiana AgCenter, 2005). The three sampling sites in Texas were selected in the middle Gulf Coast area of the State where field corn was severely infested by *D. saccharalis* during 2005 (Porter et al., 2005).

2.2. Insect culture

Field-collected larvae of *D. saccharalis* were individually reared to the pupal stage in 30-ml plastic cups (Fill-Rite, Newark, NJ) each containing approximately 10 ml of meridic diet (Bio-Serv, Frenchtown, NJ) (Huang et al., 2006b). Pupae, directly collected from fields or derived from field-collected larvae, were placed in 3.79-l cardboard cartons (Neptune Paper Products, Newark, NJ) for adult emergence, mating, and oviposition. Bioassays with field populations of *D. saccharalis* were conducted using neonates of the first, second, or third generation reared in the laboratory. A laboratory strain of *D. saccharalis* was included as a reference. The laboratory strain was originated with larvae collected from sugarcane plants near Houma in Terrebonne Parish, LA, and cultured at the USDA, ARS Sugarcane Research Laboratory, Houma, LA. This colony had been reared on a meridic diet and maintained in the laboratory for more than 20 years without exposure to any insecticides or Bt toxins.

2.3. Sources of Bt Cry1Ab protein

Two sources of Bt Cry1Ab protein were used in laboratory bioassays to determine the susceptibility of *D. saccharalis*. For the bioassays with insects collected during 2004, Cry1Ab protein used in the bioassays was extracted from Bt corn leaf tissues, whereas purified Cry1Ab protein was used for assays with insect populations derived from collections during 2005 and 2006.

To obtain Cry1Ab protein from Bt corn plants, seeds of a YieldGard® DKC69-70 corn hybrid (Monsanto, St. Louis, MO) expressing Cry 1Ab protein, and a closely related non-Bt corn hybrid, DK697 (Monsanto, St. Louis, MO), were planted in a greenhouse at the Louisiana State University Agricultural Center in Baton Rouge, LA. Cry1Ab protein was extracted from vegetative stage (V9–V12) leaf tissue of DKC 69–70 corn plants (Ritchie et al., 1993) as described in Huang et al. (2006b). Fresh leaves were removed from corn plants, cut into small pieces, and blended with distilled water in a heavy-duty blender (Model CB15, Waring Laboratory, Torrington, CT). Leaf extract solution was filtered using gauze bags to exclude coarse plant materials. The filtered solution was centrifuged and the supernatant was freeze-dried in a lyophilizer (ART, Laurel, MD) to concentrate the Cry1Ab protein. Cry1Ab concentration in the final solution was determined using an ELISA-based technique (QuantiPlate™ Kit, Envirologix, Portland, ME).

In bioassays with purified protein, Cry1Ab protein (99.99% purity) was obtained from Dr. Marianne Puztai-Carey, Department of Biochemistry, Case Western Reserve University, Cleveland, OH. The Cry1Ab protein was produced using recombinant *Escherichia coli* culture and activated with trypsin before it was used in the bioassays. The purity of Cry1Ab protein was determined using high-performance liquid chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis (Pusztai-Carey et al., 1995; Masson et al., 1998).

2.4. Insect bioassays

Susceptibility of *D. saccharalis* to purified Cry1Ab protein or Cry1Ab extracted from Bt corn leaves was determined using a modified procedure as described by Huang et al. (2006b). Neonates (<24 h old) of *D. saccharalis* were placed on a meridic diet that contained different concentrations of the Cry1Ab protein. For bioassays with corn leaf extract, Cry1Ab protein was diluted in a solution of corn leaf extract from DK697 non-Bt corn leaf tissue. The solution containing non-Bt corn leaf extract was obtained by using the same procedures as described for the Cry1Ab protein extractions. Six Cry1Ab concentrations ranging from 0.031 to 1 μg of Cry1Ab protein per g of diet (μg/g) were used in each bioassay. In bioassays with purified Cry1Ab protein, 6–8 concentrations ranging from 0.0625 to 8 μg/g were used for each test. A non-treated control (regular diet) was included in each bioassay. In addition, a negative control (diet treated with a solution containing non-Bt corn leaf extract) also was employed in the leaf extract bioassays.

In bioassays, approximately 1 ml (assays with purified Cry1Ab protein) or 1.5 ml (assays with leaf extract) of treated or non-treated diet was put into each cell of 128-cell trays (Bio-Ba-128, C-D International, Pitman, NJ). One neonate (<24 h old) of *D. saccharalis* was inoculated on the diet surface in each cell. The bioassay trays were placed in an environmental chamber maintained at 27–28 °C, 50% r.h., and an L16:D8 cycle. The number of dead larvae and surviving larvae that did not significantly increase in weight (<0.2 mg per larva) was recorded at 7 days after inoculation. Each combination of insect population by Cry1Ab concentration was replicated four times with 16–32 larvae in each replicate.

2.5. Data analysis

A measurement of 'practical' mortality (Sims et al., 1996; Huang et al., 2006b) was used to determine the relative susceptibility of different populations of *D. saccharalis*. This measurement combines both actual mortality as well as larval growth inhibition caused by Cry1Ab protein (Marçon et al., 1999). The practical mortality of *D. saccharalis* at a Cry1Ab concentration was calculated using the following equation: practical mortality (%) = 100 × [number of dead larvae + number of surviving larvae that did not show a significant gain in body weight (<0.2 mg per larva)]/total number of insects tested (Huang et al., 2006b). Larval mortality data at each Cry1Ab concentration were corrected for mortality occurring on the control diet using the method described by Abbott (1925). Corrected dose/mortality data were then subjected to probit analysis (Finney, 1971; SAS Institute, 1999) to determine Cry1Ab concentrations that caused 50% (LC_{50}) and 95% (LC_{95}) mortality and the corresponding 95% confidence intervals (CI). Relative susceptibilities of different populations of *D. saccharalis* to Cry1Ab protein were compared using LC_{50} and LC_{95} values. Lethal dose ratio tests (Robertson and Preisler, 1992) were used to determine significant differences in LC_{50}s and LC_{95}s among insect populations at the $\alpha = 0.05$ level.

3. Results

3.1. Susceptibility of D. saccharalis to Cry1Ab protein extracted from Bt corn leaf tissues

The median lethal concentrations (LC_{50}s) of Cry1Ab protein extracted from Bt corn leaf tissues to *D. saccharalis* were significantly different ($P<0.05$) among the laboratory strain and field populations collected during 2004 (Table 1). All seven field populations exhibited a lower LC_{50} value than the laboratory strain. The range of LC_{50}s among the field populations was from 0.03 to 0.32 μg/g (or ≈10-fold). The two populations collected from corn and grain sorghum in Franklin Parish were the least susceptible to the Cry1Ab protein with LC_{50}s of 0.27 and 0.32 μg/g, respectively. The LC_{50}s of these two populations were significantly greater ($P<0.05$) than the LC_{50} of the other field populations, except for the population collected from rice in Franklin Parish. The two insect populations collected from sugarcane in Avoyelles and Calcasieu Parishes were the most susceptible to the Cry1Ab protein, with LC_{50}s of 0.03 and 0.04 μg/g, respectively, which were significantly lower ($P<0.05$) than that of other populations. Significant differences ($P<0.05$) in LC_{50}s were also detected among the three other field populations, but the differences were small (<2-fold).

Difference in the 95% lethal concentrations (LC_{95}s) of Cry1Ab protein extracted from Bt corn leaf tissues to *D. saccharalis* among the eight insect populations evaluated during 2004 followed a similar pattern as observed for the LC_{50}s (Table 1). The LC_{95}s of the seven field populations were lower than that of the laboratory strain, with a range of 0.12 μg/g for the population collected from sugarcane in Avoyelles Parish to 1.18 μg/g for the Rapides population collected from corn plants.

3.2. Susceptibility of D. saccharalis to trypsin-activated purified Cry1Ab protein

Similarly, the LC_{50}s of *D. saccharalis* to trypsin-activated Cry1Ab protein were significantly ($P<0.05$) different among the laboratory strain and the 11 field populations collected during 2005 and 2006 (Table 2). All of the field populations, except one, exhibited lower LC_{50} values than the laboratory strain. The population collected from Tensas Parish during 2006 was the least susceptible to Cry1Ab protein with an LC_{50} of 0.17 μg/g, a value that was ≈6-fold higher than that of the most susceptible field population. Among the 11 field populations, three populations (Calhoun and Jackson Counties, TX, and Rapides Parish, LA) collected during 2006, were the most susceptible to the purified Cry1Ab protein, with LC_{50}s of 0.03–0.04 μg/g, followed by the two populations from Rapides Parish, LA (LC_{50} 0.06 μg/g), collected during 2005 and East Carroll Parish, LA (LC_{50} 0.07 μg/g), collected during 2006. Significant differences ($P<0.05$) in LC_{50}s also were detected among the other five field populations, but the differences were small, and ranged from 0.08 μg/g (for the population from Victoria County, TX) to 0.10 μg/g (for the population from Franklin Parish, LA, collected during 2005). Differences in LC_{50}s of the two Franklin populations collected in different years (2005 and 2006) were not significant ($P>0.05$). However, for populations collected from Rapides, Tensas, and East Carroll Parishes, LA, differences in LC_{50}s between populations collected in the 2 years at the same location were significant ($P<0.05$), but small (e.g. ⩽2-fold).

There were significant differences ($P<0.05$) in LC_{95}s among the laboratory strain and field populations of *D. saccharalis* collected during 2005–2006 (Table 2). Eight of the 11 field populations had lower LC_{95} values than the laboratory strain. The highest LC_{95} value (1.09 μg/g) among the field populations was 7-fold greater than that of the lowest (0.16 μg/g). The four populations collected from Tensas Parish, LA (2005), Rapides and East Carroll Parishes, LA, and Calhoun County, TX (2006), had the lowest LC_{95} values (0.16–0.21 μg/g), followed by the population from East Carroll Parish (2005) with an LC_{95} value of 0.30 μg/g. The two populations collected from Tensas Parish, LA, and Victoria County, TX, during 2006 had the highest LC_{50}s (1.09 and 0.96 μg/g, respectively). The differences in LC_{95}s of the other four field populations were <2-fold and were not statistically significant ($P>0.05$). The LC_{95} values for the two populations collected from the same location during successive years (2005 and 2006) were not significantly different ($P>0.05$) for the populations from Franklin and East Carroll

Parishes, LA, whereas differences between years were significant for populations from Rapides (2-fold) and Tensas (6-fold) Parishes, LA.

4. Discussion

Geographic differences in susceptibility to Bt toxins in several target pests of transgenic Bt corn have been evaluated, including *O. nubilalis* in the United States (Marçon et al., 1999; Reed and Halliday, 2001), Spain (González-Núñez et al., 2000), and Germany (Saeglitz et al., 2006); *D. grandiosella* in the mid-southern United States (Reed and Halliday, 2001, Trisyono and Chippendale, 2002); Mediterranean corn borer, *Sesamia nonagrioides* (Lefebvre), in Spain (González-Núñez et al., 2000); corn earworm, *Helicoverpa zea* (Boddie), in the United States (Siegfried et al., 2000); and western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in the US Midwestern Corn Belt (Siegfried et al., 2005). Variations in Bt susceptibility among geographical populations within an insect species, in general, were relatively small, with <7-fold differences in LC_{50}s.

Before Bt cotton was introduced, notable variation (e.g. up to 8-fold) in Bt susceptibility was observed in the tobacco budworm, *Heliothis virescens* (F.), a primary target pest of Bt cotton, across the US Cotton Belt (Stone and Sims, 1993; Luttrell et al., 1999), but field populations remained as susceptible as laboratory strains that had been reared in the absence of Bt toxins (Luttrell et al., 1999). After several years of commercial use of Bt cotton, field populations of *H. virescens* showed similar susceptibility (Hardee et al., 2001, Ali et al., 2006) to Cry1Ac protein compared with laboratory strains. Similarly, field populations of *Helicoverpa armigera* (Hübner), a primary lepidopterous target pest of Bt cotton in Asia, were susceptible to Cry1Ac protein after several years of extensive use of Bt cotton in China (Wu et al., 2006) and India (Kalia et al., 2006). Before transgenic Bt crops was commercially planted in the fields, considerable difference in Cry1Ac susceptibility (up to 198-fold) and Cry1Ab susceptibility (up to 61-fold) was reported in field populations of *H. zea* collected from southern cotton areas of the United States (Luttrell et al., 1999).

Compared with the previous reports on target pests of Bt corn, results of the current study indicate a slightly greater variation in Cry1Ab susceptibility among the seven field populations of *D. saccharalis* collected during 2004 ($\leqslant 10$-fold difference in LC_{50}s). The greater variation in Bt susceptibility among different geographical populations may suggest the potential for Bt resistance development in *D. saccharalis*. In fact, major Bt resistance alleles have been detected in two Louisiana field populations of *D. saccharalis* during 2004 and 2006 (Huang et al., 2007a; B.Y., F.H., R.L., S.M., unpublished data). However, the difference in susceptibility to purified Cry1Ab protein among the 11 populations collected during 2005–2006 was smaller than those collected during 2004, $\leqslant 6$-fold. Nevertheless, all of the 18 field populations, with one exception, exhibited relatively lower LC_{50} values than the laboratory strain. Only the field population collected from corn in Tensas Parish, LA, during 2006 had a higher LC_{50} value (1.5-fold) than the laboratory strain. The LC_{50} value (e.g. 0.12 μg/g) of Cry1Ab toxin to the laboratory strain was similar to that (e.g. 0.11 μg/g) of a strain generated from a single-pair mating of Bt-susceptible *D. saccharalis* (Huang et al., 2007b), suggesting this laboratory strain used in the current study was susceptible to the Cry1Ab toxin. In addition, except the significantly lower LC_{50} values observed for the two field populations collected from sugarcane plants, only relatively small differences ($\leqslant 6$-fold) in Cry1Ab susceptibility were detected among other crops, years, or geographic locations. The differences in Bt susceptibility among field populations of *D. saccharalis* observed here are more likely due to natural variations among populations rather than caused by selection pressure due to Bt protein exposure. As reported for other insect species, such differences in susceptibility among insect populations could be due to fitness differences (growth and development) (Rossiter et al., 1990; Marçon et al., 1999), uncontrollable variation in bioassay conditions, or other non-genetic factors (Sims et al., 1996). Therefore, the results of this study generally suggest that field populations of *D. saccharalis* remain susceptible to the Cry1Ab protein after 8 years, use of transgenic Bt corn in the region. The susceptibility data established from this study can be used as a reference to determine relative susceptibility of *D. saccharalis* to Cry1Ab protein for other geographical populations or to evaluate changes in Cry1Ab susceptibility in the future.

In the current study, the source of Cry1Ab protein used in the bioassays with the insect populations collected during 2004 was extracted from Bt corn plants. The use of Bt proteins extracted from Bt plants should better reflect the status of insect susceptibility to Bt corn compared to the use of Cry1Ab protein from recombinant *E. coli* cultures, because the Bt proteins expressed in plants are what the insects would contact in the field (National Research Council, 2002). However, the Cry1Ab protein extracted from Bt corn plants was not purified. Plant materials in the leaf extraction might affect the dose response. Therefore, tryspin-activated purified Cry1Ab protein was used in the bioassays with the insect populations collected during 2005 and 2006. This study was not designed to evaluate the difference between the uses of the two Cry1Ab sources. The current bioassays indicated an overall of 2-fold difference between the two Bt sources, suggesting the greater variation in Cry1Ab susceptibility observed among the insect populations collected during 2004 could be also due to the source of toxin.

Bioassays with both the purified Cry1Ab protein and the Cry1Ab extracted from Bt corn plants showed that the laboratory strain of *D. saccharalis* was somewhat less susceptible than the field populations. Such small, but detectable differences, could be also due to the laboratory strain being more accustomed to the meridic diet, because the laboratory strain had been reared on the meridic diet and maintained in the laboratory for more than 20 years. Similar results were also observed in *H. virescens* to Cry1Ac (e.g. up to 6-fold) (Luttrell et al., 1999) and *D. virgifera virgifera* to Cry3Bb1 (e.g. up to 6-fold) (Siegfried et al., 2005).

D. saccharalis is also an important corn pest in several other states in the mid-southern United States (Castro et al., 2004a; Porter et al., 2005). In the current study, field surveys were originally designed to evaluate Bt susceptibility in Louisiana populations of *D. saccharalis*. However, during the study, field corn was severely infested by *D. saccharalis* in the south central area of Texas. Therefore, three field populations of *D. saccharalis* were collected from this area during 2006, but the initial sampling sizes of these three populations were limited (20–30 field insects) and represented only a small area of the state. Evaluation of more field populations representing a larger geographical area in Texas and other states of the mid-southern United States is necessary.

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